Impact of chemotherapy on the ovarian reserve: Are all primordial follicles created equal?

In the study by Anderson et al. (1) published in this issue of the journal, the investigators performed a population-based national database analysis to assess family size and timescale for achieving pregnancy in women who remain fertile after they are cured of cancer. To achieve this, Anderson et al. (1) analyzed the Scottish Cancer Registry records from 1981 to 2012 linked to maternity and death records from the same country. For each subject identified, 3 controls from the population were matched. The matching was performed based on age at diagnosis, period of diagnosis by decade, previous pregnancy history, and socioeconomic status. From this database, the hazard ratio of live births was calculated. The investigators then limited the analysis to those who had at least 1 pregnancy. These patients were compared with their controls, who were adjusted for competing risks in terms of the age at live birth, family size, and period between diagnosis and the last pregnancy. The investigators found that there was a delay in achieving pregnancy in cancer survivors compared with healthy controls. This delay was expected in adult cancer survivors because they are faced with many medical, psychosocial, and economic reasons that can result in the postponement of childbearing. However, this came as a surprise to investigators assessing childhood cancer survivors because pediatric survivors have a longer period to recover from the impact of cancer diagnosis and treatment. This latter finding shows the complexity of the factors involved in family building after cancer diagnosis; many are likely to be of nonbiologic origin and heavily influenced by psychosocial issues. Surprisingly, even for an identified group of women with breast cancer who were presumed to have undergone significantly gonadotoxic chemotherapy, the timespan across which they achieved pregnancies after diagnosis was not shorter than that of age-matched controls and was even slightly longer for some diagnosis or age subgroups (1). However, the age at conception was shifted to later years in the timespan to complete childbearing.

The investigators' primary interpretation of these data was that despite cancer treatments and likely reduction of the ovarian reserve, when and if gonadotoxic chemotherapy regimens are used, the survivors had a similar "opportunity" to conceive; they speculated that this might be due to some ovarian compensation mechanisms. Let us come back to this later.

The investigators are experts with significant contributions to the field, and this population- or national registry-based study was less subject to a bias than a cohort analysis. However, the use of a national registry comes with its limitations, as acknowledged by the investigators in their own discussion section. The dataset was limited by the lack of information on infertility diagnosis and treatments. It also lacks information on fertility preservation. It is quite possible that many of the survivors had previously undergone ovarian tissue, oocyte, or embryo freezing procedures and relied on these to delay childbearing. In our experience, patients who have cryopreserved reproductive tissues tend to delay childbearing, sometimes by over a decade (2). It is also possible that some women had to receive infertility treatments, including assisted reproductive technology and egg donation, explaining the delayed, but not shortened, window of opportunity compared with that in the controls. Because the data are not applicable to early miscarriages, it is also possible that the women experienced poor reproductive outcomes, although previous data have shown that the miscarriage rates are not increased among cancer survivors. The data were also agnostic to the type of chemotherapy regimens, and hence, it is not possible to determine the actual contribution of ovarian gonadotoxicity to the picture. Another important factor that needs to be considered is that, age, not the ovarian reserve, is the key determinant of pregnancy success. This is especially true for cancer survivors, in whom pregnancies can occur with ease, even when there is severe depletion of the ovarian reserve or when there is ovarian insufficiency. This is an example of quality "compensating" for quantity (2). Bearing these limitations in mind, is compensatory recovery of the ovarian reserve possible after gonadotoxic chemotherapy?

To clinically address this question, we performed a longitudinal analysis of serum antimüllerian hormone (AMH) levels in young women who were diagnosed with invasive breast cancer and received chemotherapy or tamoxifen-only treatment (3). Longitudinal samples were collected up to 24 months after the completion of chemotherapy or, in the case of the tamoxifen-only group, during the treatment. As expected, the serum AMH levels initially dropped because AMH-producing, early-stage follicles are invariably damaged by all forms of chemotherapy. As new growth occurs from the surviving primordial follicle reserve, they begin producing AMH, leading to partial restoration of AMH levels, this time reflecting the new state of the reserve. From our ovarian transplantation experiments, we know the timeline for primordial follicles to reach antral stages to be around 3–6 months. Based on histologic data and some extrapolations of animal data, some investigators have even predicted a longer growth phase from the primordial follicle stage to the ovulatory stage. Therefore, physiologically, any recovery in the serum AMH levels beyond 12 months after chemotherapy cannot be explained by the growth of follicles from the remaining reserve. However, we found that when adjusted for age, there was no recovery beyond 12 months after chemotherapy, whereas the tamoxifen-only control group showed an age-explained decline (3). However, underscoring the critical role of DNA repair in primordial follicle survival against the chemotherapy insult, we did find that women whose oocytes were deficient for DNA double-strand break

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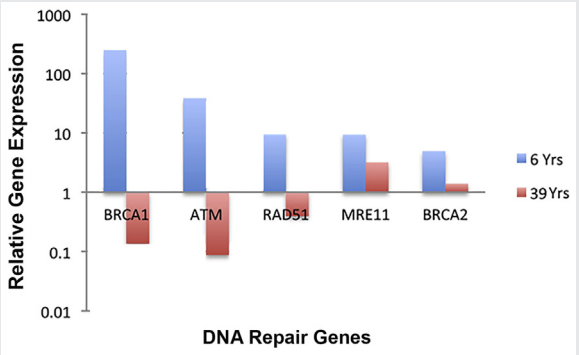
FIGURE 1



Strength of oocyte DNA repair in children vs. that in adults. Single-cell quantitative real-time polymerase chain analysis of the DNA repair genes *BRCA1*, *ATM*, *RAD51*, *MRE11* and *BRCA2* was performed using germinal vesicle stage oocytes captured from the ovarian cortex of a 6-year-old girl and a 39-year-old woman. The results suggest that children have as much as ~650 times the capacity to repair DNA double-strand breaks compared with older adults. The expression of the DNA repair genes was normalized by that of a housekeeping gene. *GAPDH* was used as the housekeeping control. The methodology has been previously described (5).

Oktay. Reflections. Fertil Steril 2021.

(DSB) repair because of *BRCA* mutations had significantly lower recovery of their ovarian reserve.

We have previously shown both in human organ culture and xenograft models that gonadotoxic chemotherapy agents, such as cyclophosphamide (an alkylating agent) and doxorubicin (a topoisomerase inhibitor), cause primordial follicle death by inducing DNA DSBs and apoptosis in primordial follicle oocytes (4). This effect is swift, causing apoptosis of the majority of primordial follicles within 12 hours of the exposure, leading to depletion of nearly 90% of the reserve within 48 hours of the exposure, in human ovarian xenograft models. In addition, especially doxorubicin can cause stromal microvascular damage and necrosis, but the contribution of microvascular damage to primordial follicle reserve loss has not been quantitated (4).

It has also recently been proposed in a mouse model that chemotherapy may deplete the ovarian reserve by causing activation of primordial follicles. Leaving methodologic issues aside (which we recently reviewed elsewhere), we were always nonplussed by this theory (4). If there is activation of primordial follicles due to exposure to chemotherapy, what happens next? Why would they all die after activation? Would we not see a large wave of follicles growing after chemotherapy exposure if this is the case? Why would the entire follicle reserve not be depleted if there is massive activation? To address the molecular mechanisms of chemotherapy-induced primordial follicle death, we developed single-primordial follicle, real-time quantitative polymerase chain reaction (PCR) and RNA sequencing approaches (5). Using our xenograft model, we exposed human ovarian tissue in vivo to cyclophosphamide or its vehicle and recovered the tissues 12 hours later. First, our histologic analysis did not show any evidence of increased entry of primordial follicles into the growth pool; if anything, we found a trend toward the opposite. Moreover, in the grafts exposed to the chemotherapy, there were increased DNA DSBs and apoptosis in the primordial follicles compared with those in the controls. In laser-captured primordial follicle oocytes from the same samples, we performed single-oocyte RNA sequencing and real-time quantitative PCR and analyzed pathways that were activated in response to the chemotherapy exposure. The study showed that the phosphoinositide 3-kinase (PI3K)/Ak strain transforming (Akt) pathway, which is involved in primordial follicle growth, was not activated. All pathway changes in the primordial follicles pointed toward a proapoptotic state in the chemotherapy-exposed primordial follicles. Interestingly, the ingenuity pathway analysis (IPA) showed that the overall pathway state favors the suppression of primordial follicle activation (5).

These findings are congruent with one of the postulates raised by Anderson et al. (1) regarding the possible slowing down of follicle loss as a compensatory mechanism. Is it possible that after a major ovarian reserve-reducing insult to the ovary, the remaining reserve is managed more economically? Unfortunately, there is no evidence based on animal models to prove this contention, and our analysis using human organ culture and xenograft models was limited to a maximum of 96 hours of timespan post-chemotherapy (5). In xenograft models and using our single-cell transcriptomic approaches, we are now exploring long-term pathway changes to determine the late effects of chemotherapy on the primordial follicle reserve and its activation. These exciting future studies will shed more light on how the ovary may economize its reserve in the face of duress.

There is also another possible explanation for the seemingly lack of compromised reproductive potential in some patients with cancer, especially those at a very young age. We have previously shown that *ATM*-mediated DNA DSB repair mechanisms in human oocytes weaken with advancing age (5). We have also shown that chemotherapy exposure activates these repair mechanisms and that some follicles may be able to repair this DNA damage and potentially recover (4). Therefore, primordial follicles are not created equally; those that have better DNA repair mechanisms survive the chemotherapy insult, whereas those with a lesser repair ability die. Because, as we have previously proposed, the DNA repair capacity of an oocyte may reflect its quality, the primordial follicles that survive chemotherapy may be of a "superior breed." This then creates a biologically plausible hypothesis to explain how quality can make up for quantity in patients with cancer and enable them to have children in delayed phases of their lives. Because we have shown that younger women and, especially, the oocytes of children have a significantly higher capacity to repair DNA damage (Fig. 1) (5), this explains why girls and younger women have a better chance of ovarian reserve "recovery." These theories are being tested in experiments that we are in the process of conducting with the support of the National Institute of Child Health and Human Development. If successful, these translational studies will lead to the development of pharmacologic approaches

to preserve the primordial follicle reserve via enhancement of primordial follicle DNA DSB repair mechanisms.

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